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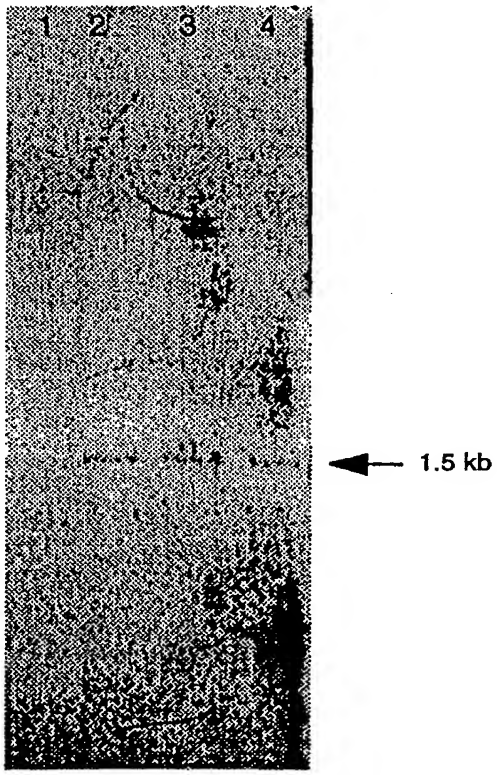
(54) Title: IMPROVED STARCHY FLOURS

(57) Abstract

The present invention is directed to a starchy flour comprising a modified protein content. The starchy flour is capable of producing a dough that, when compared with a corresponding wild–type starchy flour, comprises an increased pasting temperature, an increased peak viscosity, and more stability upon heating and cooling. Preferably the starchy flour is obtained from a root tuber species including potato, sweet potato, cassava, beet, yam, artichoke and turnip, or a combination thereof. This invention is also directed to a modified starchy flour prepared from maize. The modified starchy flour is obtained by transforming a plant with at least one gene construct that encodes a matrix protein. The matrix protein is preferably selected from the group consisting of glutenin, gliadin, albumin, and globulin.



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IMPROVED STARCHY FLOURS

The present invention relates to improved starchy flours. More specifically, this invention is directed to improved starchy flours comprising modified protein content.

BACKGROUND OF THE INVENTION

Wheat storage proteins represent the major source of human dietary proteins in the world (1). The sequential extraction and solubilization of these storage proteins produces albumin, globulin and gliadin/glutenin fractions, the latter fraction accounting for 10% of total soluble proteins (2,3). According to their relative mobility on SDS-PAGE, glutenins have been subdivided into high-molecular-weight (HMW) and low-molecular-weight (LMW) subunits (4).

Glutenins and gliadins, the constitutive proteins of gluten, are water-insoluble polymers. They form inter-molecular cross-links, thus creating a proteinaceous matrix which provides bread dough with unique viscoelastic properties (1). Glutenins play a more prominent role than gliadins in creating this viscoelastic matrix (5) and evidence is now available to show that both HMW and LMW glutenins contribute to the overall functional properties of dough (4,3,6). In wheat, the role of LMW glutenins in determining dough quality was first indicated by correlations established between allelic variation and dough properties (7), and more recently by the positive effect of a selection based on LMWG genes (8). New wheat lines with altered protein composition have now been obtained through genetic engineering (9,10,11), and overexpression of a HMW glutenin subunit resulted in improving the functional properties of bread dough (12).

Root-tuber crops such as potato and sweet potato also represent a major source of dietary protein worldwide (13,14). Compared to wheat, they can be grown with relatively high yields in low-input agricultural systems, and this characteristic has contributed to increase their popularity in poor rural communities in Africa, South East

Asia and China where they represent the main food source (13). Root tuber crops are best suited for direct consumption in both industrialized and autarcic communities. However, unlike proteinaceous flours, the starchy flours derived from root tuber crops have low viscosity and elasticity. As a result, such flours have found limited applications in the processing industry.

The main interest for using potato flour in food is to benefit from its high water-holding capacity (15,16). However, there has been no demonstration of improved quality of starchy flours within the prior art. This invention is directed to improved physical properties of starchy flours, including increased pasting temperature, peak viscosity, and stability on heating and cooling. Within the prior art, attempts have been made to improve the quality of starchy flours through the modification of the polysaccharidic backbone of the starch molecules with limited success. However, no modifications have been made regarding the protein content of the flour. This invention is directed to the improvement of the functional properties of starchy flours through the modification of protein content.

It is an object of the invention to overcome disadvantages of the prior art.

The above object is met by the combinations of features of the main claims, the sub-claims disclose further advantageous embodiments of the invention.

SUMMARY OF THE INVENTION

The present invention relates to improved starchy flours. More specifically, this invention is directed to improved starchy flours comprising a modified protein content.

According to the present invention there is provided a starchy flour comprising a modified protein content. This invention is also directed to the starchy flour as defined above, wherein a dough prepared from the starchy flour, when compared with a corresponding wild-type starchy flour, comprises an increased pasting temperature, an increased peak viscosity, and more stability upon heating and cooling.

This invention also embraces the starchy flour as defined above, wherein the starchy flour is obtained from a root tuber species. Preferably the root tuber species is selected from potato, sweet potato, cassava, beet, yam, artichoke and turnip, or a combination thereof. This invention is also directed to a starchy flour as defined above, wherein the starchy flour is prepared from maize.

This invention also relates to the starchy flour as defined above, wherein the modified protein content is obtained by transforming a plant with at least one gene construct that encodes a matrix protein. The matrix protein is preferably selected from the group consisting of glutenin, gliadin albumin, and globulin.

This invention is also directed to a method of preparing an starchy flour with a modified protein content, comprising: transforming a plant with at least one gene construct that encodes a matrix protein, selecting a transformed plant comprising the gene construct; and harvesting the starchy flour. Preferably the at least one gene construct comprises a gene that encodes a matrix protein selected from the group consisting of glutenin, gliadin, albumin, and globulin.

Starchy flours are typically characterized as having low viscosity and no elasticity. This invention describes the isolation of a low-molecular-weight glutenin gene (LMWG-MB1) from wheat, downstream ligation of the LMWG-MB1 to a patatin promoter for tuber-specific expression, and transfer of the construct to potato leaf explants through *Agrobacterium sp.* infection. Furthermore, a potato line exhibiting high expression of *lmwg*-mb1 peptide was propagated and transferred to field plots. Flour obtained from these field-grown tubers exhibit from about a 2 to about a 4 fold increase in viscosity compared to flour from non-transgenic tubers. Detailed analysis of dough properties show that high accumulation of *lmwg-mb1* result in significant increases in pasting temperature, peak viscosity and stability upon heating (95° C) and cooling (50°C).

The present invention demonstrates that a transgenic potato line is capable of producing a hydrated flour with improved rheological properties and that improved starchy flours from potato or other starchy flour-producing plant can be used as substrates for applications requiring increased viscosity and heat stability, for example as improved thickeners for the food industry (dehydrated sauces, pie fillings, soup mixes, baby foods, etc.)..

This summary of the invention does not necessarily describe all necessary features of the invention but that the invention may also reside in a sub-combination of the described features.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

FIGURE 1 shows a Southern blot analysis of transgenic potato DNA. Twenty μg of DNA from a non-transgenic line (lane 1) and from transgenic lines Ben1, Ben 9 and Ben16 (lane 2,3,4 respectively) were digested with HindIII-EcoRI, separated by electrophoresis on a 0.8% agarose gel, vacuum-transferred onto a nylon membranes (Hybond, Amersham) and hybridized with clone LMWG-MBI as probe.

FIGURE 2 shows total RNA was isolated from tubers of non-transgenic (lane 1) and transgenic lines Ben1, Ben 9 and Ben16 (lanes 2,3,4 respectively). Ten μg of total RNA was loaded in each well and separated by electrophoresis. The northern blot was hybridized with clone LMWG-MB1 as probe.

FIGURE 3 shows proteins extracted from tubers of non-transgenic lines (lane 1) and transgenic lines Ben16 (lane 2) were separated by electrophoresis, electrotransferred onto a nitrocellulose membrane (Bio-Rad), and immunodetected using an anti-g-gliadin polyclonal antibody (1/400 dilution).

DESCRIPTION OF PREFERRED EMBODIMENT

The present invention relates to improved starchy flours. More specifically, this invention is directed to improved starchy flours comprising modified protein content.

The following description is of a preferred embodiment by way of example only and without limitation to the combination of features necessary for carrying the invention into effect.

By "starchy flour" as used herein, it is meant the flour produced by any root tuber species, such as, but not limited to, potato, sweet potato, cassava, maize, beet, yam, artichoke, turnips and the like.

Wheat gluten proteins are classified in two main groups, gliadins and glutenins, glutenins playing the major role in determining the functional properties of dough prepared from hydrated flour. Gluten-like proteins are absent from starchy flours. Without wishing to be bound by theory, it is possible that this is one reason why humid doughs made from starchy flours have extremely low viscosity and no elasticity.

By "matrix protein" as used herein it is meant a protein that when introduced within a starchy flour, increases the pasting temperature, peak viscosity and stability of the dough may. It is preferred that the matrix protein is capable of interacting with the polysaccharide matrix of a starchy flour. It is also preferred that the matrix protein is capable of forming inter-molecular cross-links, thereby creating a proteinaceous matrix. Matrix proteins may also form water insoluble polymers. It is to be understood that the matrix protein of the present invention may also include more than one protein, as combinations of proteins may also exhibit desired property of creating a viscoelastic matrix of dough. Examples of matrix proteins include, but are not limited to, both low and high molecular weight glutenins, gliadins, albumins and globulins.

In a preferred embodiment of the present invention, a low-molecular weight glutenin gene is expressed in potato tubers; this modification results in the modification of the rheological properties of the humid dough. However, it is to be understood that the present invention is not restrictive to the use of a glutenin gene as any other suitable gene, capable of encoding a matrix protein that increases the pasting temperature, peak viscosity and stability of the dough may be used. It is preferred that the matrix protein is capable of interacting with the polysaccharide matrix of a starchy flour.

Furthermore, this invention is not limited to any specific plant, and may for example include, but not be restricted to, potato, sweet potato, cassava, beet, yam, artichoke, turnip, or other plant from which a starchy flour may be obtained.

The present invention is also not limited to a specific transformation procedure or a specific DNA construct, as other constructs comprising genes encoding matrix proteins may also be used following the method of the present invention. Rather, the present invention is directed to the use of at least one recombinant protein which is capable of interacting with the polysaccharidic matrix in such a way that the overall properties of the resulting starchy flour, paste or dough are modified.

By "regulatory element" or "regulatory region", it is meant a portion of nucleic acid typically, but not always, upstream of a gene, and may be comprised of either DNA or RNA, or both DNA and RNA. Regulatory elements may include those which are capable of mediating organ specificity or temporal gene activation. Furthermore, "regulatory element" includes promoter elements, core promoter elements, elements that are inducible in response to an external stimulus, or elements that are activated constitutively In the context of this disclosure, the term "regulatory element" also refers to a sequence of DNA, usually, but not always, upstream (5') to the coding sequence of a structural gene, which includes sequences which control the expression of the coding region by providing the recognition for RNA polymerase and/or other

factors required for transcription to start at a particular site. An example of a regulatory element that provides for the recognition for RNA polymerase or other transcriptional factors to ensure initiation at a particular site is a promoter element. A regulatory element may also include those elements located downstream (3') to the site of transcription initiation, or within transcribed regions, or both.

There are several types of regulatory elements, including those that are tissue dependant, developmentally regulated, inducible and constitutive. A regulatory element that is tissue dependant, or controls the differential expression of a gene under its control, is activated within certain organs or tissues of an organ of a plant. The gene constructs of the present invention preferably incorporate the use a tissue dependant regulatory element so that the gene of interest encoding a matrix protein is expressed in a tissue dependant manner, for example within the tuber tissue of a plant. Any tuber-specific regulatory element may be used for the purposes as described herein. However, inducible regulatory elements, or in some instances constitutive regulatory elements may also be used if desired.

An inducible regulatory element is one that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor, that binds specifically to an inducible regulatory element to activate transcription, is present in an inactive form which is then directly or indirectly converted to the active form by the inducer. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, herbicide or phenolic compound or a physiological stress imposed directly by heat, cold, salt, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. A plant cell containing an inducible regulatory element may be exposed to an inducer by externally applying the inducer to the cell or plant such as by spraying, watering, heating or similar methods.

A constitutive regulatory element directs the expression of a gene throughout the various parts of a plant and continuously throughout plant development. Examples of known constitutive regulatory elements include promoters associated with the CaMV 35S transcript. (Odell et al., 1985, *Nature*, **313**: 810-812), the rice actin 1 (Zhang et al, 1991, *Plant Cell*, **3**: 1155-1165) and triosephosphate isomerase 1 (Xu et al, 1994, *Plant Physiol.* **106**: 459-467) genes, the maize ubiquitin 1 gene (Cornejo et al, 1993, *Plant Mol. Biol.* **29**: 637-646), the *Arabidopsis* ubiquitin 1 and 6 genes (Holtorf et al, 1995, *Plant Mol. Biol.* **29**: 637-646), and the tobacco translational initiation factor 4A gene (Mandel et al, 1995 *Plant Mol. Biol.* **29**: 995-1004).

This invention is therefore directed to gene constructs comprising a regulatory element in operative association with a gene of interest, wherein the gene of interest encodes a matrix protein for example, but not limited to, low and high molecular weight glutenins, gliadins, albumins and globulins. It is preferred that a tissue specific regulatory element, for example, but not limited to a tuber-specific regulatory element, be used to direct expression of the matrix protein within tuber tissue. In this manner, gene constructs of the present invention may be introduced into a plant that produces a root tuber, so that a starchy flour comprising a exogenous matrix protein may be obtained.

The constructs of the present invention can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, micro-injection, electroporation, etc. For reviews of such techniques see for example Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academy Press, New York VIII, pp. 421-463 (1988); Geierson and Corey, *Plant Molecular Biology*, 2d Ed. (1988); and Miki and Iyer, Fundamentals of Gene Transfer in Plants. In *Plant Metabolism*, 2d Ed. DT. Dennis, DH Turpin, DD Lefebrve, DB Layzell (eds), Addison Wesly, Langmans Ltd. London, pp. 561-579 (1997). The present invention further includes a suitable vector comprising the chimeric gene construct.

To aid in identification of transformed plant cells, the constructs of this invention may be further manipulated to include plant selectable markers. Useful selectable markers include enzymes which provide for resistance to an antibiotic such as gentamycin, hygromycin, kanamycin, and the like. Similarly, enzymes providing for production of a compound identifiable by colour change such as *GUS* (β-glucuronidase), or luminescence, such as luciferase are useful.

This invention also embraces propagules obtained from a plant transformed with the gene construct as defined above. Example of propagules include stem cuttings, tuber sections, seeds and the like.

Furthermore, the present invention relates to a transgenic root tuber comprising a matrix protein.

As described in the examples, the accumulation of a recombinant matrix protein, such as glutenin, in tubers alters the functional properties of a starchy flour such as potato flour. These altered properties were also observed in tubers obtained from transgenic plants grown in the field

The protein comprising starchy flours of the present invention can be used within applications that require increased viscosity and heat stability, for example, but not limited to, thickeners for the food industry (dehydrated sauces, pie fillings, soup mixes, baby foods, etc.) or starchy flours suitable for pastry- or bread-making.

The above description is not intended to limit the claimed invention in any manner, furthermore, the discussed combination of features might not be absolutely necessary for the inventive solution.

The present invention will be further illustrated in the following examples. However it is to be understood that these examples are for illustrative purposes only, and should not be used to limit the scope of the present invention in any manner.

Experimental Protocol

Plant transformation and construct

The coding region of clone LMWG-MB1 (19) was subcloned as a XbaI-SacI fragment in vector pBlue Script (Stratagene). This subclone was inserted into the SmaI-SacI sites of vector pBI-101.3 (Invitrogen), producing pBI/LMWG. A patatin promoter was amplified with the use of primer A: GCTTTGTTTTATTATCATTTTTCACAC (SEQ ID NO:1) and B: TTGGATCGAAACTCGTATATTGTTCGTACC (SEQ ID NO:2) using genomic DNA from potato var. Kennebec (20) as template. PCR was performed as described by Benmoussa et al. (19). The amplicon was first subcloned into pBluescript and then inserted into pBI/LMWG as a SalI-SmaI fragment, creating pBI/LMWG/pat. This chimaeric construct was introduced into *Agrobacterium tumefaciens* LBA4404 by electroporation as described by Khoudi et al. (21). After 3 days of pre-cultivation of potato leaf explants in MS medium (22), the *Agrobacterium* cells harboring pBI/LMWG/pat were used to inoculate sterile explants (15 min at room temperature). Individual explants were removed and briefly pampered on sterile filter papers. Transformed potato cells were allowed to grow on a MS medium containing 50 mg/l kanamycin, 500 mg/l carbenicilin, 1 mg/l benzyladenine and 0.1 mg/l naphthalene acetic acid. The explants were transferred to fresh selection medium every 10 days. After a 3-week periods in the selection medium, the calli were excised and transferred to an organogenesis medium (MS containing 0.1 mg/l zeatin, 2 mg/l benzyladenine, 50 mg/l kanamycin and 500 mg/l carbenicilin). The regenerated shoots were transferred to rooting medium (MS + 50 mg/l kanamycin). Rooted plantlets were grown to maturity in the greenhouse.

Nucleic acids analysis

Genomic DNA was isolated from the leaves of transgenic and non-transgenic lines according to Rogers and Bendich (23). Twenty micrograms of DNA were digested with HindIII-EcoRI, separated by electrophoresis on a 0.8% (w/v) agarose gel and vacuum-transferred to a Nylon membrane (Hybond-N Amersham). Total RNA was isolated from tubers as described by de Vries et al. (24). Ten μg of total RNA were separated on 1% agarose formaldehyde gels in MOPS (3-Morpholino propane sulfonic acid) buffer and blotted to a Nylon membrane (Hybond-N Amersham). Both blots were pre-hybridized at 68°C in 2X SSC, 5X Denhardt's, 0.5% SDS, and 100 μg/ml of salmon sperm DNA for 3 hr. Hybridization was performed in the same solution overnight with a ^{32}P-labeled LMWG-MB1 XbaI-SacI fragment. After hybridization, the filters were washed four times with 2X SSC and 0.5% SDS at 68°C for 40 min.

Protein analysis

One gram of tuber (FW) was ground with a mortar pestle in 4 ml of SDS-PAGE sample buffer (50 mM tris, pH 8.3, 3% (w/v) SDS, 2% (v/v) b-mercaptoethanol, 10% glycerol and 0.01% (w/v) bromophenol blue. The homogenates were heated at 60°C for 30 min as suggested for solubilization of gluten proteins (17), and centrifuged at 13000 g for 15 min. Protein content was determined in aliquots of supernatant extracts using the Bio-Rad DC Protein Assay reagent (25). Approximately 100 μg of total proteins from each sample were denatured at 100°C for 3 min. SDS-PAGE was performed according to Laemmli (26) in 12% (w/v) acrylamide gels, and proteins were electrotransfered onto nitrocellulose membranes (Bio-Rad).

Nitrocellulose blots were blocked for 1 hr in TBS (Tris-base 0.019 M, NaCl 0.5M at pH 7.5) containing 5% skimmed milk, and washed twice in TBS-Tween 20 (0.5 ml Tween 20/l TBS) for 10 min. Blots were incubated at room temperature under

Ashton Research Station, University of Bristol, UK). After two washes for 15 min in TBS-Tween 20, blots were incubated for 1 hr in the buffer solution described above containing 2% skimmed milk and a peroxydase-conjugated goat anti-rabbit IgG (1/1000 dilution). Following binding of the secondary antibody, the blots were washed twice in TBS-Tween 20, and revealed by using the BM chemiluminescence system of Boehringer Manheim at room temperature.

Rheological properties

Plants from one non-transgenic line and transgenic lines Ben 9 and 16 were propagated first by stem cuttings and then with tuber sections. These plants were transferred to field in early summer. All tubers were harvested in early fall and kept in the dark at 4°C for three months. Tubers were then lyophilized and ground to flour with a Waring blender (Dynamic Corporation of America, New Hartford, CT). The apparent viscosity was determined on sifted potato flour using a modified AACC method (27). One hundred gram of floor in 200 g of water were placed in a 500 ml-Erlenmyer. In triplicate, apparent viscosity (centipoises) was determined randomly at 23°C with spindle #5 at 20 rpm (RV model, Brookfield Engineering Laboratories Inc., Stoughton., MA).

Amylograph viscosity curves were determined on 35 g of potato flour in 500 ml of phosphate-citrate buffer (pH 5.3-5.35). The mixture was heated from 50°C to 95°C (1.5°C/min), followed by hold at 95°C for 30 min and cooling at 50°C, according to AACC method 22-10 (28).

Plant transformation

A LMW glutenin gene was introduced into potato leaflets by *Agrobacterium* mediated DNA transfer. Fifty developing shoots were picked from the margin of forty leaf pieces following co-cultivation and regeneration on selection medium. From these, thirty were allowed to root in vitro and propagated. These kanamycin-resistant lines

leaf pieces following co-cultivation and regeneration on selection medium. From these, thirty were allowed to root in vitro and propagated. These kanamycin-resistant lines were first tested for the presence of the transgene by PCR amplification. All kanamycin-resistant lines proved to be PCR positive. Southern analysis was used to demonstrate stable integration of the constructs. Since the restriction sites used for digest were within the construct, a single 1.5 kb band was found in transgenic plants (Fig. 1). The HindIII/EcoRI probe did not hybridize with DNA from non-transgenic plants. Northern analysis was performed on total RNA extracted from tubers of transgenic and non-transgenic plants. As shown in Figure 2, hybridization yielded a single mRNA band in transgenic plants, its size corresponding to that expected for the LMWG-MB1 transcript (Fig. 2). The northern analysis also showed a wide variation in the level of transcript between individual transgenic lines.

Protein analysis

A polyclonal anti-g-gliadin antibody was used for immunodetection; it had demonstrated sufficient reactivity and specificity to detect LMW 1D1 glutenin subunits in transgenic insect cells (17). The autoradiogram shows the presence of one band at 35 KDa in transgenic potato plant samples (Fig. 3), which corresponds to the molecular weight deduced from the amino acid sequence of clone LMWG-MB1. No such band was found in blots of proteins from untransformed plant samples (Fig.3, Lane 1). Cross-reacting material was found at 46 KDa in homogenates from transgenic and non-transgenic plants, suggesting the presence of a g-gliadin homolog in potato tubers. Two transgenic lines (Ben 9 and 16) were selected from the thirty initial transgenic lines for further analysis based on their ability to accumulate low (Ben 9), and high (Ben 16) levels of the the *lmwg-mb1* peptide in tubers. Transfer to the greenhouse produced mature plants with tubers, which were used for propagation Four cycles of growth and tuber production were used to bring transgenic populations to a size suitable for transfer in field conditions. Southern analysis showed that constructs containing the LMWG-MB1 gene were integrated into genomic DNA in all transgenic lines.

The effect of LMW glutenin accumulation

Field-grown tubers from transgenic and non-transgenic lines were harvested, lyophilized and ground to flour. Flour samples from each plots were then divided in sub-samples, and randomly tested for viscosity in a 1/2 (w/v) water mix. Mean viscosity of suspensions from transgenic lines Ben 9 and 16 was estimated to 1330 and 1766 centipoise respectively, compared to 533 centipoise for the non-transgenic controls. Detailed analysis of dough properties also showed that high accumulation of *lmwg-mb1* in Ben 16 resulted in significant increases in pasting temperature, peak viscosity and stability upon heating (95° C) and cooling (50°C) (Table 1). Heated hydrated flour samples from transgenic line Ben 9 were not significantly different from controls.

Table 1: Viscosity[a] and Brabender pasting properties[b] of flour samples from transgenic and non-transgenic potato plants (var. Kennebec).

Lines	Viscosity at 23°C (cp)	Paste Temperature (°C)	Viscosity at 95°C (BU)	Viscosity at 95°C; Hold 30 min (BU)	Viscosity at 50 °C (BU)
(non transgenic)	533±153	61.5±0	590±10	410±10	550±10
Ben 9 (transgenic)	1300±100	70.1±15	500±20	390±10	555±15
Ben 16 (transgenic)	1767±57	65.3±0	665±5	460±20	605±15

[a]Determined at 23°C with a Brookfield viscosimeter on a flour/water homogenate (1/2, w/w).

[b]Determined with a Visco/amylograph using 35 g of flour/500 ml of citrate-phosphate buffer at pH 5.3, 1.5°C per min heating rate and 30 min hold at 95°C.

These results demonstrate that the accumulation of a recombinant matrix protein, such as glutenin, in tubers alters the functional properties of a starchy flour such as potato flour. Furthermore, this alteration of properties was maintained in plants grown within the field, under normal cultural practices. These results also indicate that *lmwg-mb1* subunits can create an homogeneous proteinaceous matrix that increases viscosity, without the contribution from HMW glutenin or gliadin-type peptides. However, it is within the scope of the present invention that the LMW glutenin may be used in conjunction with other proteins such as HMW glutenin or gliadin-type proteins, or other non-storage proteins providing that beneficial properties, including increased pasting temperature, peak viscosity, and stability upon heating or cooling of the resultant flour are obtained.

Without wishing to be bound by theory, these results demonstrate that the recombinant LMW glutenin may have created cross-links with other proteinaceous components of the homogenates. It is also possible that carbohydrates and lipids present in the homogenates interacted with recombinant LMWG subunits as suggested by (18).

The present results demonstrate that recombinant *lmwg-mb1* can accumulate in potato tubers and that selection for high accumulation of *lmwg-mb1* yields a transgenic potato line capable of producing a hydrated flour with improved rheological properties. These observations indicate that improved starchy flours from potato or maize, could be used as substrates for applications requiring increased viscosity and heat stability, for example as improved thickeners for the food industry (dehydrated sauces, pie fillings, soup mixes, baby foods, etc.). These results also indicate that similar strategies could be used to provide alternatives to current uses of starchy tubers in the poorest segments of communities in Africa, Southeast Asia, and China. Improved expression and accumulation of glutenin genes could result in the production of starchy flours suitable for pastry- or bread-making.

All citations listed herein are incorporated by reference.

The present invention has been described with regard to preferred embodiments. However, it will be obvious to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as described herein.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OF PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A starchy flour comprising a modified protein content.

2. A method of preparing an starchy flour with a modified protein content, comprising:

   i) transforming a plant with at least one gene construct that encodes a matrix protein,

   ii) selecting a transformed plant comprising said at least one gene construct; and

   iii) harvesting said starchy flour.

3. The starchy flour of claim 1, wherein a dough prepared from said starchy flour, when compared with a corresponding wild-type starchy flour, comprises;

   an increased pasting temperature;
   an increased peak viscosity; and
   more stability upon heating and cooling.

4. The starchy flour of claim 1, wherein said starchy flour is obtained from a root tuber species.

5. The starchy flour of claim 1, wherein said root tuber species is selected from the group consisting of potato, sweet potato, cassava, beet, yam, artichoke and turnip, or a combination thereof.

6. The starchy flour of claim 1, wherein said starchy flour is prepared from maize.

7. The starchy flour of claim 1, wherein said modified protein content is obtained by transforming a plant with at least one gene construct that encodes a matrix protein.

8. The starchy flour of claim 7, wherein at least one of said matrix protein is selected from the group consisting of glutenin, gliadin, globulin, and albumin.

9. The starchy flour of claim 8, wherein said matrix protein is a glutenin

10. The starchy flour of claim 9, wherein said glutenin is encoded by LMBG-MB1.

11. The method of claim 2, wherein said at least one gene construct comprises LMWG-MB1.

12. A transgenic tuber producing plant comprising a gene construct, said gene construct comprising a regulatory region in operative association with a gene of interest, said gene of interest encoding a matrix protein.

13. The transgenic potato plant of claim 12, wherein said matrix protein is selected from the group consisting of glutenin, gliadin, globulin, and albumin.

14. The transgenic potato plant of claim 12, wherein said gene construct comprises LMWG-MB1.

15. A propagule obtained from the transgenic potato plant of claim 12.

16. The propagule of claim 15 comprising a seed.

17. The propagule of claim 15 comprising a stem cutting.

18. The propagule of claim 15 comprising a tuber section.

19. A tuber obtained from the transgenic potato plant of claim 12.

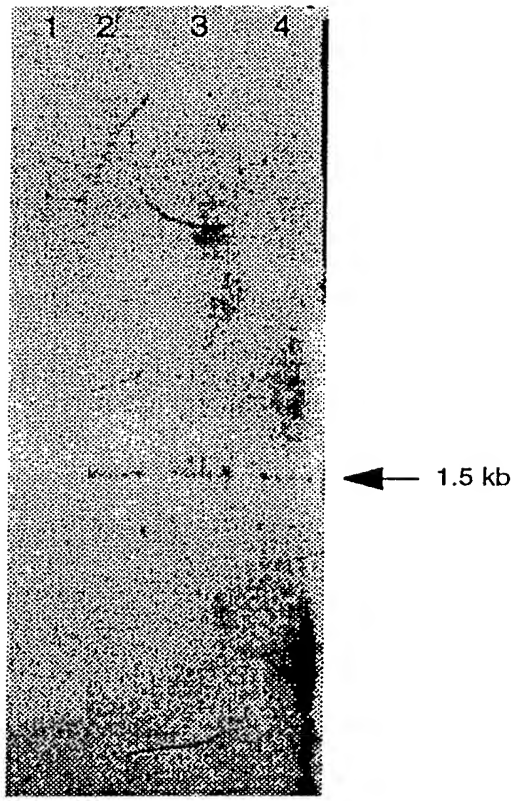

FIGURE 1

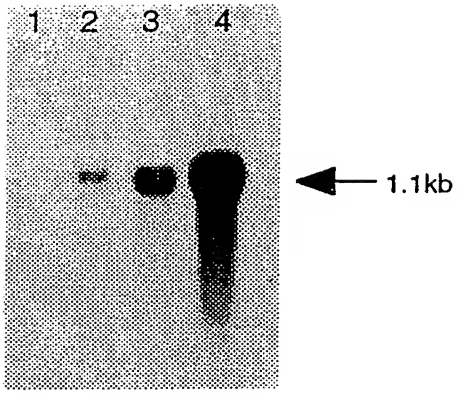

FIGURE 2

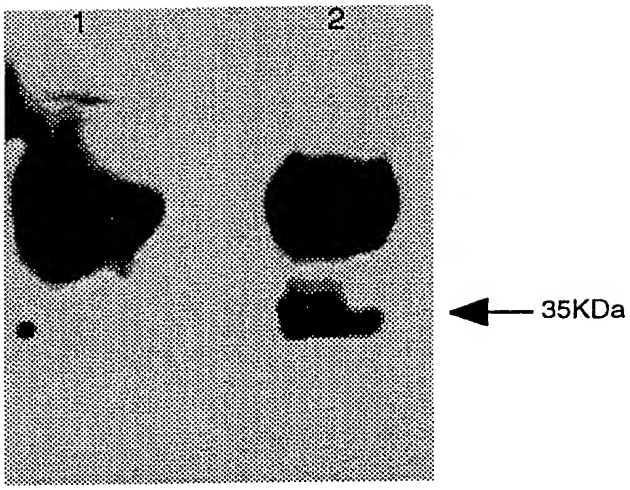

FIGURE 3

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Yelle, Serge
Simard, Ronald
Vezina, Louis
Laberge, Serge

<120> Improved Starchy Flours

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/CA 99/00870

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/29 C12N15/82 A01H5/00 A01H5/10 A23L1/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CIACCO, C.F., ET AL.: "Characterization of starches from various tubers and their use in bread baking" CEREAL CHEMISTRY, vol. 54, no. 5, 1977, pages 1096-1108, XP000877362 the whole document ---	1,3-5, 7-10
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2000	22/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

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INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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